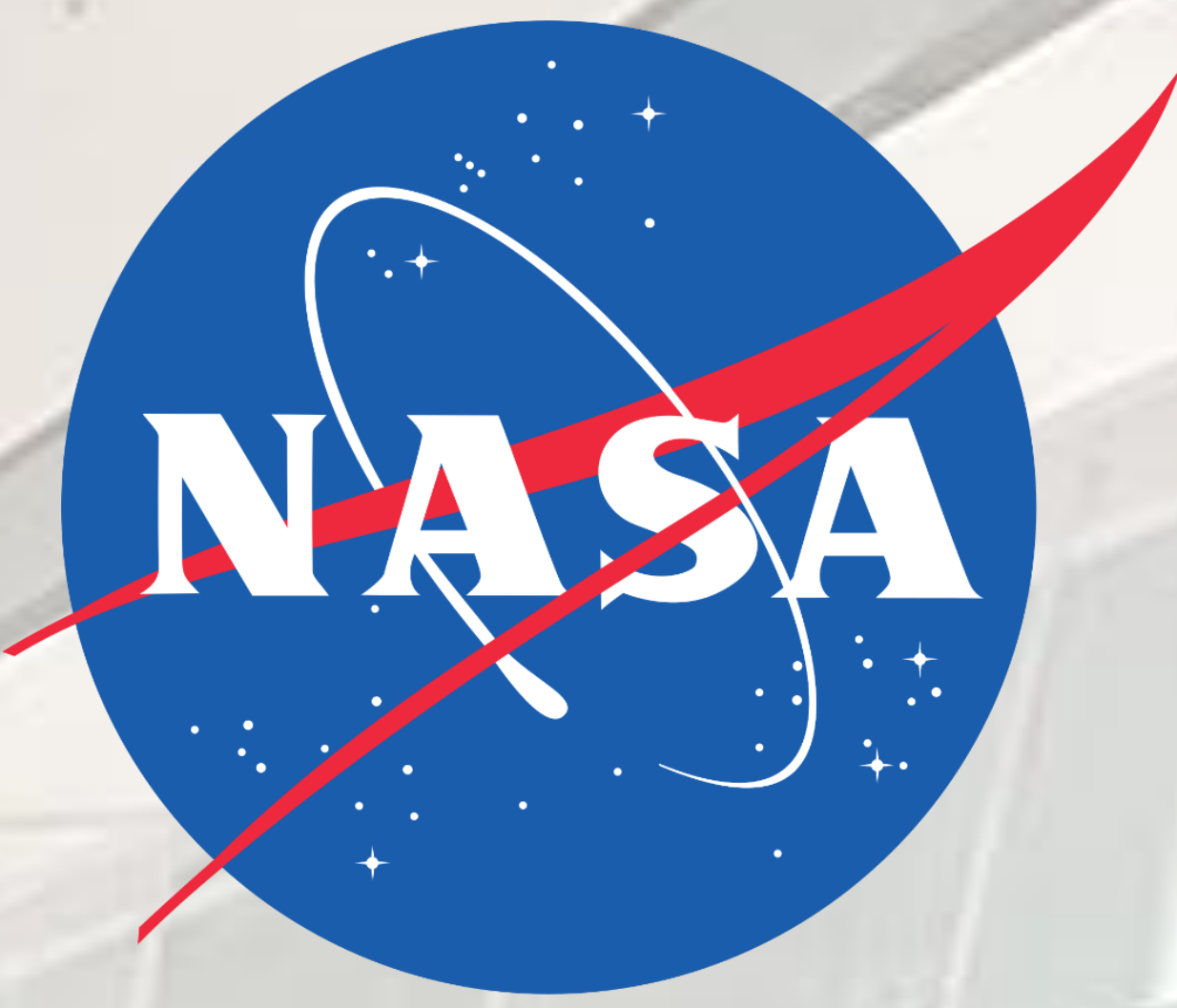


# Conditions of the Toll-like Receptors System of Human Innate Immunity Cells During Long-term Isolation

Cody L. Gutierrez<sup>1</sup>, Stephanie Krieger<sup>2</sup>, Satish Mehta<sup>1</sup>, Sergey A. Ponomarev<sup>3</sup>, and Brian Crucian<sup>4</sup>

<sup>1</sup>JES Tech, Houston, Tx, <sup>2</sup>KBR, Houston, Tx, <sup>3</sup>Institute of Biomedical Problems, Moscow, Rus, <sup>4</sup>NASA Johnson Space Center, Houston, Tx



## Introduction

Long duration isolation is widely used by NASA as an analog for space flight with previous missions including several Antarctic bases, an undersea research station (NEEMO), and other isolation chambers. ‘SIRIUS’ is a multi-compartment pressurized facility at the IBMP in Moscow, Russia, in which subjects will spend 8 months executing tasks similar to those performed by astronauts in space. Prolonged isolation studies like SIRIUS replicate many of the influences expected to occur during long duration spaceflight missions including isolation, stress, confinement, and living in remote conditions. The immune study performed on SIRIUS mission was led by Dr. Sergey Ponomarev at the Institute of Biomedical Problems in Moscow, Russia. The NASA JSC Immunology lab will provide analysis of immune cell function, serum cytokines and stress hormones, and salivary cortisol, cytokines, and viruses.

## Objectives

1. Investigate the effect of isolation for 8 months at the Russian NEK spaceflight vehicle-simulation on immune dysregulation consisting of cellular function, salivary cytokine profiles, and basic peripheral leukocyte distribution.
2. Investigate the effect of NEK deployment on the reactivation and shedding of latent herpesviruses.
3. Correlate all findings with the Russian assessment of novel innate immunity parameters to derive a comprehensive assessment of the effect of prolonged isolation on human immunity and associated clinical risks.

## Hypothesis

1. That mission-like isolation in the Russian NEK facility, consisting of circadian misalignment, isolation, stress, and ‘station lifestyle’, will result in a pattern of immune dysregulation similar to that observed in astronauts onboard ISS.
2. Immune changes will correlate with the reactivation of latent herpesviruses (which persists during orbital flight), as measurable adverse clinical manifestation or with other observed symptomology.

## Methodology

Blood and saliva will be collected from 6 volunteers, 3 male and 3 female, pre-, in-, and post isolation (see sampling matrix). From these samples, multiple assays and immune functions will be tested:

- **Peripheral Mononuclear Cell Isolation and Preservation:** Blood PBMCs will be isolated in Russia by density gradient centrifugation
- **T Cell Function:** T cell activation will be induced by mitogenic stimulation. Cells will be then assessed for the induction of activation markers on specific T cell subsets by flow cytometry.
- **Saliva/Plasma Cytokine Concentrations:** The concentration of immune-modulating cytokines in plasma/saliva will be assessed by multiplex array.
- **Salivary Latent Herpesvirus DNA:** The presence of viral DNA will be assessed in passive drool saliva samples via PCR.
- **Salivary Cytokine Profiles:** Up to 13 cytokines will be assessed by cytometric bead multiplex array. The Immunology Laboratory has previously generated normal range data for these analytes.



## Expected Results

The SIRIUS-21 mission began in November of 2021 and successfully concluded with the crew emerging in July of 2022. Currently, the logistics of shipping the samples from Russia to the United States is being organized. Once samples are received expectations are that this study will validate the Russian analog using flight validated measures provided by NASA. The novel aspects of the study may also yield new mechanistic associations that can shed light on the causes of stress-associated immune alterations. Further, this data will allow a direct flight/ground comparison, such that NEK deployment may be evaluated as an appropriate location to test potential immune countermeasures. Finally, the generated data will serve as an excellent control for past and current immune investigations onboard ISS and future Artemis missions.



## Acknowledgements

Image Credit: NASA 2017

## Sampling Matrix

ASSAY		TIMEPOINTS														
BLOOD CELLS	Blood cell distribution	IBMP	IBMP	IBMP	IBMP	IBMP	IBMP	IBMP			IBMP		IBMP		IBMP	IBM
	Blood cell count	IBMP	IBMP	IBMP	IBMP	IBMP	IBMP	IBMP			IBMP		IBMP		IBMP	IBM
	Blood cell TLR assessment	IBMP	IBMP	IBMP	IBMP	IBMP	IBMP	IBMP			IBMP		IBMP		IBMP	IBM
	Blood Cell (PBMC) functions	NASA	NASA	NASA	NASA	NASA	NASA	NASA			NASA		NASA		NASA	NASA
	Serum HSP/HMGB	IBMP#	IBMP#	IBMP#	IBMP#	IBMP#	IBMP#	IBMP#			IBMP#		IBMP#		IBMP#	IBM#
BLOOD SERUM	Serum cytokines	IBMP*	IBMP*	IBMP	IBMP	IBMP	IBMP	NASA			NASA		NASA		NASA	NASA
	Serum stress hormones	IBMP*	IBMP*				IBMP	IBMP			NASA		NASA		NASA	NASA
SALIVA	Saliva cortisol	IBMP*	IBMP*	IBMP	IBMP	IBMP	IBMP	NASA			NASA		NASA		NASA	NASA
	Saliva viruses	NASA(3)	NASA(3)	NASA(3)	NASA(3)	NASA(3)	NASA(3)	NASA(3)			NASA(3)		NASA(3)		NASA(3)	NASA
	Saliva cytokines	NASA	NASA	NASA	NASA	NASA	NASA	NASA			NASA		NASA		NASA	NASA
		-7	-3	+3	+7	+14	+21	+2	+3	+4	+5	+6	+7	+8	+9	
Day		Day						Month								Day
Baseline		Isolation Period														